INVESTIGATION OF PUTATIVELY SECRETED ENZYMES FROM *SCHISTOSOMA MANSONI* AS POTENTIAL VACCINE CANDIDATES: CLONING, EXPRESSION AND PURIFICATION

Araujo BO, Tararam CA, Farias LP, Leite LCC

Biotecnologia Molecular IV, Centro de Biotecnologia, Instituto Butantan, São Paulo, São Paulo, Brazil

Schistosomiasis is a chronic disease that causes nearly 20 000 deaths per year. The development of a vaccine against this disease is a priority for the developing world. The information obtained from the *Schistosoma mansoni* transcriptome opened a window of opportunity for the development of novel vaccine candidates. From these, we have selected three genes, alkaline phosphatase, carboxypeptidase and sphingomyelinase, for investigation as vaccine candidates, due to their putative role in the parasite's physiology and likely surface location exposed to the host's immune system. We first obtained the full sequence of alkaline phosphatase (missing the 5' region) using 5' RACE. We then cloned the genes, excluding their predicted signal sequence/transmembrane regions, from the parasite's mRNA using RT-PCR, sequencing, digestion and ligation into the appropriate vectors. Transformation in three *E. coli* strains and induction of expression were carried out, and evaluated by SDS-PAGE and Western Blot. The antigens were obtained as inclusion bodies in *E. coli*, requiring solubilization with different concentrations of urea, followed by in-vitro refolding and purification by nickel affinity chromatography. We achieved different levels of recovery after purification, being the alkaline phosphatase the best purified. Immunization of rats is underway in order to produce antibodies for immunolocalization of these proteins in the different parasite stages.

Acknowledgement: We thank FAPESP for financial support.

Key words: alkaline phosphatase, carboxypeptidase, protein purification, *Schistosoma mansoni*, sphingomyelinase